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HUMAN PROSTATE CANCER PROGRESSION MODELS AND THERAPEUTIC INTERVENTION

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Our laboratory has developed two cellular models of human prostate cancer progression. The LNCaP prostate cancer progression model is based upon the well-known cellular interaction between human prostate or bone stromal cells and LNCaP cells *in vivo*. The marginally tumorigenic LNCaP cells acquired tumorigenic and metastatic potential upon cellular interaction with either prostate or bone fibroblasts. A subline termed C4-2 was observed to grow readily in castrated animals and acquired metastatic potential spreading from the primary tumor site to the lymph node, the seminal vesicles, and the axial skeleton, resulting in an intense osteoblastic reaction. The second model is ARCaP, where prostate cancer cells derived from the ascites fluid of a man with metastatic disease exhibited an Androgen- and estrogen-Repressed Prostate Cancer cell growth and tumor formation in either a hormone-deficient or a castrated environment. However, the growth of either the tumor cells *in vitro* or the tumors *in vivo* was suppressed by both estrogen and androgen. While the tumor cells expressed low levels of androgen receptor and prostate-specific antigen (PSA), they were highly metastatic when inoculated orthotopically. Distant metastases to a number of organs were detected, including the liver, lung, kidney, and bone.

We have employed a human prostate cancer progression model as a system to study the efficacy of gene therapy. Results of the study show that whereas universal promoters, such as Cytomegalovirus (CMV) and Rous Sarcoma Virus (RSV) promoter-driven tumor suppressors (eg. p53, p21, and p16), were effective in inhibiting prostate tumor growth, the advantages of driving the expression of therapeutic toxic genes using a tissue-specific promoter prostate-specific antigen (PSA) and a tumor- but not tissue-specific promoter, osteocalcin (OC), are preferred. In the case of the PSA promoter, we can achieve cell-kill in PSA-producing human prostate cancer cells. To circumvent the supporting role of bone stroma for prostate cancer epithelial growth, we have recently developed a novel concept where the expression of therapeutic toxic genes is driven by a tumor- but not a tissue-specific OC promoter. Osteocalcin-thymidine kinase (OC-TK) was found to efficiently eradicate the growth of osteosarcoma, prostate, and brain tumors both *in vitro* and *in vivo*. We observed that androgen-independent human prostate cancer cell lines expressed OC-TK at higher levels than androgen-dependent human prostate cancer cell lines. We have obtained data to suggest that Ad-OC-TK plus a pro-drug acyclovir (ACV) may be used as an effective therapy to treat prostate cancer bone metastasis in models where the growth of androgen-independent PC-3 and C4-2 tumors in the bone has occurred.

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Key word : Prostate cancer, Progression model, Gene therapy, Osteocalcin promoter, PSA promoter

INTRODUCTION

Prostate cancer is the leading cancer diagnosed in U.S. men and the third leading cause of cancer death in U.S. men¹⁾ Despite recent efforts to improve treatment of advanced disease using chemotherapy or to prevent human prostate cancer development by the alteration of dietary habits, both incidence and deaths from prostate cancer have risen consistently. In view of the long latency (25-35 years) of prostate cancer before reaching the clinical stage, new therapeutic strategies need to be implemented, both to stage men with prostate cancer and to offer novel treatment therapies in men who harbor metastatic

prostate cancer. In our laboratory, we decided first to establish a relevant human prostate cancer model for laboratory investigation. Second, we have developed a molecular staging program to clone novel genes that can be used as molecular markers to differentiate indolent from virulent forms of prostate diseases. Third, we have developed a molecular therapeutic program to devise novel therapeutic approaches to treat human prostate cancers. In this report, we have summarized our recent efforts in the development of animal models to study prostate cancer growth and progression, and the development of an adenoviral therapeutic gene delivery system for the treatment of androgen-independent metastatic

Table 1. ARCaP cell phenotypes in mice closely mimicked behaviors in the patient

	In Mice	In Patient
Gene Expression		
PSA	+	+
AR	+	+
Neuroendocrine factors	+	+
Metalloproteinases	+	ND
Metastasis		
Bone	+	+
Kidney	+	+
Lung	+	+
Pancreas	+	ND
Adrenal	+	ND
Ascites fluid	+	+

prostate cancers in experimental models.

PROSTATE CANCER MODEL DEVELOPMENT

Our laboratory is responsible for the development of two human prostate cancer xenograft and tissue culture model systems using either LNCaP or newly developed androgen-repressed human prostate cancer cells (ARCaP), grown either *in vitro* or *in vivo*. LNCaP cells, a marginally tumorigenic human prostate epithelial cell line, when administered with either prostate or bone fibroblasts, were promoted to grow and disseminate in castrated hosts. Through this manipulation, we successfully developed a series of human prostate LNCaP sublines that share a cell lineage relationship with the parental LNCaP cells. The sublines C4-2 and C4-2 B acquired both androgen independence and tumorigenicity as well as metastatic potential to the skeleton. We have observed that these cell lines, when administered either subcutaneously or orthotopically, can progress from their primary site of growth to the lymph node and eventually to the bone with a significant delayed period (average 6.8 months)²⁾. To understand whether this model may be used efficiently for assessing prostate cancer bone interaction, we have recently inoculated either parental LNCaP or its sublines directly to the skeleton and observed the growth of prostate cancer cells in the bone⁵⁾. In a series of studies, we have demonstrated that although LNCaP failed to grow in the bone, C4-2 and C4-2 B were observed to grow successfully in the skeleton. We have observed time-dependent increases of PSA in serum elaborated by prostate cancer cells grown in the bone. In concordance with the data obtained from patients, we have seen that despite elevation of serum PSA following prostate cancer growth in the bone, circulating tumor cells in serum had no correlation with serum PSA. This observation is in agreement with the clinical observation that serum PSA bears no relationship to circulating prostate cancer cells in the blood³⁾. In a recent publication,

Hyttinen et al.⁴⁾ demonstrated that progression-associated prostate cancer genetic modifications can be observed upon prostate cancer progression. Stage- and progression-specific genetic alterations were induced in LNCaP and its lineage-derived cell lines, suggesting that genetic changes of prostate cancer cells may be subjected to epigenetic cues from the surrounding stromal cells.

ARCaP represents an androgen-repressed human prostate cancer cell line derived from the ascites fluid of a man with metastatic prostate cancer. The patient was considered to have rapid progressive and invasive disease. Results obtained from experimental studies of ARCaP cells in mice closely mimicked the clinical characteristics of the ARCaP cells in the patient⁵⁾. Table 1 lists the characteristics of ARCaP cells in the patient and the behaviors of ARCaP cells in athymic mice. ARCaP cells expressed low levels of androgen receptor and PSA, and appeared to be invasive and metastatic when inoculated orthotopically in the animal⁵⁾. As in the original patient, ARCaP cells in nude mice metastasized to the bone, kidneys, lungs, pancreas, and adrenal glands with high levels of metalloproteinase expression. This cell line may represent the further progression of prostate cancer from its androgen-refractory or -insensitive state toward androgen repression^{5,6)}. Androgen-repressed human prostate cancer can be viewed as a positive responder to intermittent androgen therapy and potentially sensitive to flutamide withdrawal therapy. In both cases, we hypothesized that circulating androgen, or estrogen derived from aromatization of either endogenous or exogenous androgens, and growth factor-receptor pathways that may be activated upon flutamide withdrawal can significantly attenuate the growth of prostate cancer cells that exhibit androgen- and estrogen-repressed phenotypes. ARCaP cells failed to amplify their androgen receptors and appear to have no structural mutations at either DNA- or steroid-binding domains. This suggests the following three mechanisms of androgen suppression mediated by the androgen receptor: 1) Androgen receptor mutation at exon 1, 2) Interaction between androgen receptor and other interactive proteins, and 3) Influencing the growth of prostate cancer cells by epigenetic factors, such as factors secreted by host microenvironment and stroma which affect tumor angiogenesis and attenuate host immune responses to the growing tumors. Presently, we do not know the mechanisms for androgen-induced repression of prostate cancer growth and metastasis.

Table 2 summarizes the models developed to study human prostate cancer progression. The parental LNCaP and its lineage-derived cell lines C4, C4-2, and C4-2 B (and C4-2 B sublines) are a series of cell lines with varied androgen dependency and

Table 2. Progression of human prostate cancer from an androgen-dependent to an androgen-repressed state

Cell line Phenotype	LNCaP Androgen-dependent →	C4 Androgen-independent	C4-2/C4-2 B>	ARCaP Androgen-repressed
Androgen stimulation	+++	+	—	—
Androgen repression	—	—	+	++
AR expression	++	++	+	+
PSA expression	+	++	+++	+
Invasion/metastasis	—	—	+	++

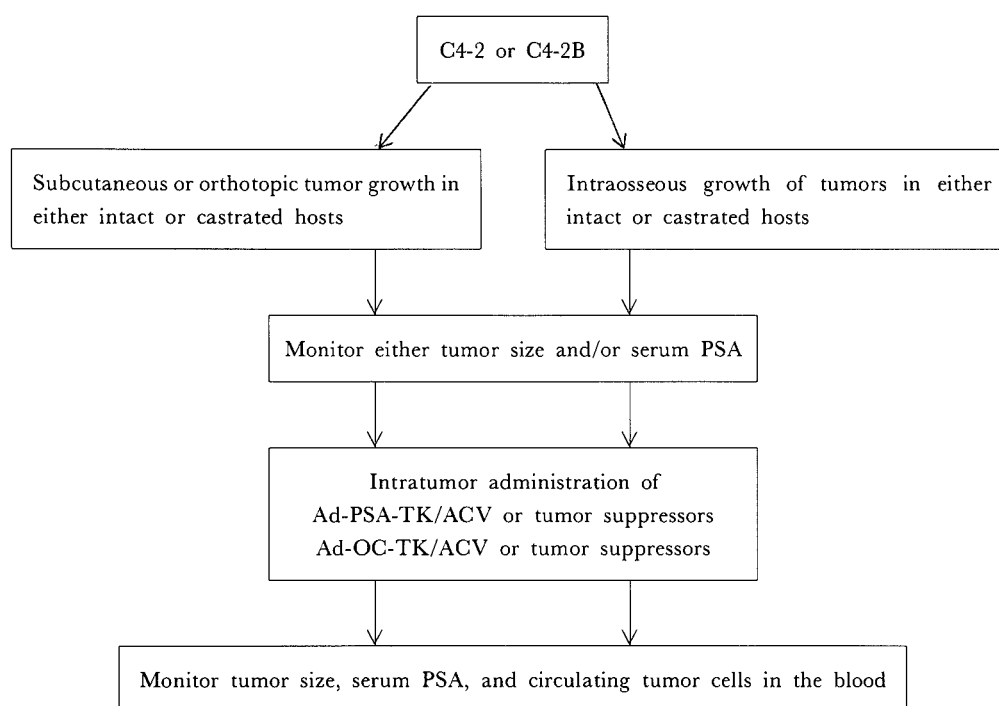


Fig. 1. Pre-clinical gene therapy protocol for the treatment of human prostate cancer metastasis.

metastatic potential. Upon culturing LNCaP cells under androgen-deprived conditions for a long period, the repression of a LNCaP cell clone by androgen was observed⁷⁾; the repressed LNCaP cell clone can grow in castrated males under the support of Matrigel, a reconstituted basement membrane. These results are consistent with the concept that androgen-responsive LNCaP cells can be "triggered" to exhibit androgen repression under the influence of altered cell culture conditions. ARCaP, a human prostate cancer cell line derived from an orchiectomized patient with highly invasive disease, represents the end stage of prostate cancer. Based upon these observations, we propose that prostate cancer progressed from the androgen-dependent to the androgen-independent state through cancer cell-stromal interaction. By manipulating the growth conditions of the cancer cells (eg. androgen deprivation), prostate cancer may progress further to become androgen and estrogen repressed. The repressed clone (s) may acquire additional invasive and metastatic potential, which could be lethal to the

host.

GENE THERAPY

In view of the rapid progress in understanding the molecular and biological aspects of prostate cancer, much effort has recently been devoted to exploring the possible uses of gene therapy to control prostate cancer growth, differentiation, apoptosis, and senescence. Our laboratory was among the first to use adenoviruses to deliver the therapeutic toxic gene thymidine kinase and tumor suppressors (p53, p21, and p16) to eradicate the growth of androgen-independent prostate cancer tumors in experimental models^{8,9)}. We observed that tumor suppressors p53, p21, and p16 and the toxic gene thymidine kinase were effective in inhibiting the growth of androgen-independent human prostate cancer cells both *in vitro* and *in vivo*. Significant survival and much reduced tumorigenicity was found by the use of tumor suppressors delivered by a universal promoter CMV. In these studies, we have observed that among the tumor suppressors, p53 appears to be the most potent

inhibitor of prostate tumor growth. p16 in turn had a greater tumor suppressor effect than p21. Tumor suppressors appear to be more potent when inhibiting the growth of smaller rather than larger tumors⁹⁾ These results, taken together, suggest that tumor suppressors and toxic genes can block growth, induce apoptosis, and cause cycle arrest of androgen-independent growth of human prostate cancer cells in animal models.

To improve the delivery and expression of therapeutic tumor suppressors or toxic genes in human prostate cancer cells, we have recently developed tissue-specific promoter-driven therapeutic genes and expressed them in localized and metastatic prostate cancers. In this effort, we have explored the possibility of using the PSA promoter as a delivery system to express toxic genes in androgen-dependent as well as androgen-independent LNCaP lineage-derived cell lines C4-2 and C4-2 B. This model system is believed to be relevant to human prostate cancer progression for the following reasons: 1) LNCaP expressed PSA and PSMA, and its growth is stimulated by dihydrotestosterone both *in vivo* and *in vitro*; and 2) The LNCaP-derived cell line C4-2 expressed high levels of PSA in the absence of testicular androgen^{2,10)} This cell line appears to be androgen-independent and exhibits metastatic potential when inoculated orthotopically²⁾ C4-2 cells were observed to metastasize from the primary tumor to lymph nodes, and eventually to distant bone sites with marked osteoblastic reactions. This model was chosen for our studies in part because of the expression of a useful human prostate cancer marker PSA; this mimics the human prostate cancer condition where advanced androgen-independent prostate cancer expressed high levels of PSA. Using this experimental model, we have clearly demonstrated that an adenovirus, Ad-PSA-TK, which contains a long PSA promoter enhancer (p61) TK, expressed high levels of TK activity and inhibited the growth of both PSA-producing androgen-dependent LNCaP and androgen-independent C4-2 cell lines *in vitro* and tumor growth *in vivo*. Ad-PSA-TK, however, was ineffective in inhibiting the growth of a human transitional cell carcinoma WH growth *in vitro*. These observations clearly demonstrated that the PSA promoter has the advantage of direct delivery of therapeutic genes to cells or tissues. One advantage of using the p61 PSA promoter to deliver therapeutic genes to prostate cancers is that this promoter can elicit high transactivating activity for therapeutic toxic genes in androgen-refractory human prostate cancer without resorting to the administration of exogenous androgen. This strategy can overcome the controversial limitations of androgen administration to men with metastatic prostate cancer.

Another approach we have developed is the effective use of the osteocalcin (OC) promoter to drive the expression of therapeutic toxic genes in human prostate cancer. Ad-OC-TK was shown to eradicate the growth of both rat and human osteosarcomas *in vivo* and *in vitro*^{11,12)} Ad-OC-TK was found to be expressed in a number of human calcified malignant diseases, including both androgen-dependent and androgen-independent prostate cancer, brain, mammary, and lung cancers. Using the OC promoter-driven therapeutic toxic gene delivery, we have accomplished the dual effect of eradicating the growth of prostate cancer cells while in the bone as well as their supporting osteoblasts. In an experimental model of human prostate cancer growth in bone, we demonstrated that the delivery of Ad-OC-TK together with the pro-drug ACV significantly impaired the growth of androgen-independent human prostate cancer cell lines C4-2 and PC-3 both *in vitro* and *in vivo*. These findings were supported further by the histomorphologic examinations of the tumors in the skeleton and x-rays of the bone lesions before and after treatment. Fig. 1 lists the general strategies of gene therapy for the treatment of prostate cancer metastasis.

SUMMARY AND FUTURE PERSPECTIVES

We have developed a number of useful and unique animal models for the study of human prostate cancer growth and progression. These models are useful in analyzing markers that are indicative of prostate cancer progression and can be employed as a powerful system to study the potential genetic alterations associated with cancer progression and rational therapeutic approaches to block the growth of cancer cells *in vivo*. We have developed several classes of therapeutic adenoviral constructs, p53, p21, and p16 tumor suppressors, and TK/ACV for bystander cell-kill, all of which have been observed to inhibit the growth of human prostate cancer experimental models. In addition, we have employed tissue-specific promoters, including PSA and OC, to deliver and express high levels of therapeutic genes in tumor epithelium and/or its supporting stroma. This approach of targeting both the prostatic epithelium and the stroma will open new avenues for the treatment of prostate cancer bone metastasis.

Future directions in prostate cancer research rely upon the further investigation of the molecular mechanisms which signal prostate cancer growth, differentiation, apoptosis, and senescence. It is important, however, to focus on the clinically relevant targets, which unfortunately are poorly defined today. In view of the lack of knowledge of cancer biology and metastasis, additional efforts may lead to a more comprehensive understanding of the molecular

pathways of cell signaling during prostate cancer progression. Close interaction between molecular/cell biologists and the surgeons of future generations ("molecular" surgeons) will lead to new strategies for the management of advanced prostate cancer.

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ヒト前立腺癌の進行モデルと新しい治療法

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我々はヒト前立腺癌の進展に関した2つの細胞モデルを開発した。LNCaP 前立腺癌進展モデルは、生体内での前立腺または骨の間質細胞と LNCaP 細胞との相互作用に基づいており、これによって腫瘍形成能と転移能を獲得したものである。派生株 C4-2 は去勢動物で容易に発育し、リンパ節、精囊腺、骨に転移する。次のモデル ARCaP は、癌性腹水由来のヒト前立腺癌細胞で、アンドロゲンおよびエストロゲンによって増殖を抑制され、去勢下で腫瘍を形成した。ARCaP はアンドロゲン受容体および PSA を低レベルで発現し、同所移植によって肝、腎、骨などに高頻度で転移した。

これらのモデルを用いて遺伝子治療の研究を行った。CMV または RSV プロモーターのようなユニバーサル・プロモーターで p53, p21, p16 といった癌抑制遺伝子を発現させると癌の発育を抑えるのに効果的であったが、組織特異的 PSA プロモーターや腫瘍

特異的オステオカルシン (OC) プロモーターで細胞傷害性の遺伝子を発現させる方が優れていると思われる。PSA プロモーターの場合、PSA 産生ヒト前立腺細胞で細胞死をもたらす。骨間質細胞が前立腺上皮の増殖を促す効果の逆を考え、我々は最近、腫瘍特異的であるが組織特異的でない OC プロモーターで細胞傷害性の遺伝子を発現させるという新しい概念を展開させた。オステオカルシン チミジンキナーゼ (OC-TK) は *in vitro*, *in vivo* で骨肉腫、前立腺、脳の腫瘍を効果的に一掃した。また、アンドロゲン依存性のヒト前立腺癌細胞株よりも非依存性のヒト前立腺癌細胞株の方が OC-TK の発現が高いことを我々は見出しており、Ad-OC-TK とプロドラッグであるアシクロビル の投与は、アンドロゲン非依存性の PC-3 や C4-2 腫瘍が骨で増殖するという前立腺癌の骨転移に対し効果的な治療法となるであろう。

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